REMARKS

These remarks are in response to the Office Action mailed April 10, 2001. Applicant affirms election of Group II, claims 54-73, with traverse, in a telephonic restriction on March 27, 2001. New claims 123-155 have been added. No new matter has been added. Claims 72 and 73 have been canceled without prejudice. Thus, upon entry of the amendment, claims 54-71 and 123-155 are under examination.

Title and the Abstract

Applicant has amended the title and the abstract as requested by the Examiner.

Drawings

Applicant notes the Examiner's concern regarding Figures 1, 4, and 14-16. Applicants will submit formal drawings upon allowance of the claims.

Claim Objection

Applicant has rewritten claim 57 with the correct spelling of the term "epoxide", thereby rendering this objection moot.

REJECTIONS UNDER 35 U.S.C.§112

Applicants respectfully request that the rejection under 35 U.S.C. §112, second paragraph be withdrawn.

Claim 54 has been amended to provide the term "recombinant cell" since the cell need not be recombinant.

Claim 54 has been amended to recite "detectable marker" in line 5, rather than selectable marker.

Claim 67 has been amended to recite detectable markers which are "expressable".

Claims 72 and 73 have been canceled, thereby rendering any confusion as to these claims moot.

Claim 70 has been amended to recite that the "cell component is a transducing protein", thereby rendering any lack of antecedent basis moot.

REJECTIONS UNDER 35 U.S.C. §§102 and 103

The rejection of claims 54, 55, 58, 62, 63, 67, 69, and 73 as rejected under 35 U.S.C. §102 as allegedly anticipated by Stover et al. U.S. Patent 5,679,515, hereinafter, the '515 patent is respectfully traversed.

Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration (In re Spada, 15 USPQ 2d 1655 (Fed. Cir. 1990), In re Bond, 15 USPQ 2d 1566 (Fed. Cir., 1990).

The "microenvironment" described by the '515 patent refers to a host cell that is capable of infection by mycobacterium, an intracellular prokaryote. Exemplary microenvironments in the '515 patent would include macrophage-type cells or macrophages, which are infected by mycobacterium.

In contrast, Applicant's "microenviroment" does not consist of host cells and infection by the "recombinant cell", but rather encapsulation or engulfment by molecular structures as discussed on page 45, lines 12-21 of the specification, for example. Such molecular structures include gel microdrops (GMDs) which are comprised of a biocompatible matrix.

"Microenvironment", as described on page 50, line 18 bridging to page 51, line 5, is any molecular structure which provides an appropriate environment for facilitating the interactions necessary for the method of the invention. An environment suitable for facilitating molecular interactions include, for example, liposomes prepared from a variety of lipids

including phospholipids, glycolipids, steroids, long-chain alkyl esters; *e.g.*, alkyl phosphates, fatty acid esters; *e.g.*, lecithin, fatty amines and the like. A mixture of fatty material may be employed such a combination of neutral steroid, a charge amphiphile and a phospholipid. Illustrative examples of phospholipids include lecithin, sphingomyelin and dipalmitoylphosphatidylcholine. Representative steroids include cholesterol, cholestanol and lanosterol. Representative charged amphiphilic compounds generally contain from 12-30 carbon atoms. Mono- or dialkyl phosphate esters, or alkyl amines; *e.g.*, dicetyl phosphate, stearyl amine, hexadecyl amine, dilauryl phosphate, and the like.

Different types of encapsulation strategies and compounds or polymers can be used with the present invention. For instance, high temperature agaroses can be employed for making microdroplets stable at high temperatures, allowing stable encapsulation of cells subsequent to heat-kill steps utilized to remove all background activities when screening for thermostable bioactivities. Encapsulation can be in beads, high temperature agaroses, gel microdroplets, cells, such as ghost red blood cells or macrophages, liposomes, or any other means of encapsulating and localizing molecules. The present invention does not rely on infection by a recombinant cell or organism. The creation of the microenvironment of the present invention is for localizing molecules in such a way that they are in close enough proximity to interact with one another.

In one preferred embodiment, the molecule is derived from an environmental sample, e.g., a mixed population of organisms. A recombinant host cell expressing such molecules while encapsulated, is co-encapsulated with a target cell in order to determine the effect on that cell (e.g., effect on cell growth) or to determine an effect on a target cell component. The present invention does not require infection resulting in intracellular localization of a recombinant reporter cell. The '515 patent does not teach or describe expression of such bioactive molecules derived from mixed populations of organisms. In fact, the '515 patent does not teach or describe co-encapsulation of a drug and the reporter cell, as shown in column 14, lines 19-48. A mycobacterial reporter strain is used to infect cultured cells and then the cultured cells are treated

with drugs for 1-14 days. The effect of the drug on the recombinant reporter strain is then observed. In sharp contrast, the methods of the present invention require co-encapsulation of both the bioactive molecule and the "reporter strain" (i.e., the target cell or recombinant cell) so that they directly interact. The molecule is not contacted with the microenvironment externally, as in the '515 patent.

The '515 patent fails to teach such encapsulation techniques or microenvironments and therefore cannot anticipate the present invention. Accordingly, Applicant requests withdrawl of the rejection under §102.

The rejection of claims 54, 55, 58, 59, 62, 63, 67, 68, 69, and 73 under 35 U.S.C. §103 as allegedly obvious in view of Stover et al. U.S. Patent 5,679,515, hereinafter, the '515 patent, in view of Valdivia et al. and Trias et al., U.S. Patent 5,989,832, hereinafter, the '832 patent, is respectfully traversed.

Stover, the '515 patent is discussed above. Stover specifically uses mycobacterial reporter strains since they infect host cells, i.e., they become intracellular. Valdivia is cited as teaching the use of GFP in the study of host pathogen interactions. Trias is cited for teaching a method of screening for agents that modulate the activity of non-tetracycline efflux pumps of prokaryotic cells. Trias is also cited as teaching the use of bacteria that can naturally exist within host eukaryotic cells, such as Salmonella and Mycobacteria and form a microenvironment as a result of infection of the eukaryotic cell.

The failure of the '515 patent to teach the microenvironments and screening system of the claimed invention cannot be remedied by the addition of Valdivia or Trias, alone or in combination. The '515 patent does not teach encapsulation-it describes infection by the recombinant cell utilized in the examples (i.e., mycobacterium). Valdivia merely provides a label or marker that is commonly used in studies of protein-protein interactions, nucleic acid-

protein interactions, and the like. Trias provides a disclosure that is somewhat cumulative to the '515 patent in that it focuses on the ability of a bacterium to infect a cell, thus forming a microenvironment for study of the bacterium and host-pathogen interactions. Trias fails to teach or suggest use of any microenvironment, e.g., a microdroplet, a liposome, a macrophage, for localizing molecules such that they interact in a confined environment. In addition, as discussed above, the preferred source of the molecule that affects a target cell component or cell growth is a mixture of organisms and more preferably from an environmental source, which is not taught nor suggested by Trias alone or in combination with any of the cited references.

Accordingly, Applicants respectfully request that the rejections under 35 U.S.C. §103 be withdrawn.

CONCLUSION

In summary, for the reasons set forth herein, Applicants maintain that claims 54-71 and 123–155 clearly and patentably define the invention, respectfully request that the Examiner reconsider the various grounds set forth in the Office Action, and respectfully request the allowance of the claims which are now pending.

If the Examiner would like to discuss any of the issues raised in the Office Action, Applicant's representative can be reached at (858) 677-1456. Please charge any additional fees, or make any credits, to Deposit Account No. 50-1355.

Respectfully submitted,

Date: October 10, 2001

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Version with Markings to Show Amendments

IN THE SPECIFICATION:

Please delete the present title and substitute therefor:

--HIGH THROUGHPUT SCREENING METHOD FOR IDENTIFICATION OF BIOMOLECULES--

Please delete the present abstract and substitute therefor:

The present invention provides a method of screening for an agent that modulates the activity of a target cell component, wherein the target cell component and a selectable marker are expressed by a cell. The method includes co-encapsulating the agent in a microenvironment with the cell expressing the target cell component and detectable marker and detecting the effect of the agent on the activity of the cell component. In one embodiment, the agent is an enzyme or small molecule. The invention also provides a method for enriching for target DNA sequences containing at least a partial coding region for at least one specified activity in a DNA sample. The method includes a) co encapsulating in a micro-environment a mixture of target DNA obtained from a mixture of organisms with a mixture of DNA probes comprising a detectable marker and at least a portion of a DNA sequence encoding at least one enzyme having a specified enzyme activity; b) incubating the co-encapsulated mixture under such conditions and for such time as to allow hybridization of complementary sequences; and c) screening for the specified activity.

Please delete the present priority data on page 1 and enter the following rewritten priority data:

This application is a continuation and claims the benefit of priority under 35 USC § 120 of U.S. application Serial No. 09/098,206, filed June 16, 1998, now issued as US

6,174,673, which is a continuation-in-part of U.S. Patent Application No. 08/876,276, filed June 16, 1997.

IN THE CLAIMS:

Please amend the claims as follows:

54. (Amended) A method of [screening for an agent] identifying a molecule that modulates the activity of a target cell component [, wherein the target cell component and a selectable marker are expressed by a cell, the method] comprising co-encapsulating the [agent] molecule and a cell expressing a target cell component of interest and a detectable marker, in a microenvironment [with the recombinant cell expressing the target cell component and detectable marker and detecting the] under conditions that allow the molecule and the target cell component to interact, and detecting an effect of the [agent] molecule on the activity of the cell component, thereby identifying a molecule that modulates the activity of the target cell component.

55. (Amended) The method of claim 54, wherein the [agent] molecule is an enzyme or small molecule.

56. (Amended) The method of claim 54, wherein the [agent] molecule is derived from a gene expression library.

57. (Amended) The method of claim 55, wherein the enzyme is selected from the group consisting of lipases, esterases, proteases, glycosidases, glycosyl transferases, phosphatases, kinases, mono- and dioxygenases, haloperoxidases, lignin peroxidases, diarylpropane peroxidases, [epozide] epoxide hydrolases, nitrile hydratases, nitrilases, transaminases, amidases, and acylases.

58. (Amended) The method of claim 54, wherein the [agent] molecule inhibits the activity of the target cell component.

59. (Amended) The method of claim 54, wherein the [agent] molecule enhances the activity of the target cell component.

60. (Amended) The method of claim 54, wherein the [agent] molecule is expressed from a recombinant cell co-encapsulated with the [recombinant] cell expressing the target cell component and detectable marker.

63. (Amended) The method of claim 54, wherein the micro-environment is a liposome, gel microdrop, bead, agarose, cell, macrophage, or ghost cell.

67. (Amended) The method of claim 54, wherein the detectable marker is a [fluorescent dye, a visible dye,] a bioluminescent molecule, a chemiluminescent molecule, a radioactive material, or an enzymatic substrate.

70. (Amended) The method of claim 54, wherein the [protein] cell component is a transducing protein.

Please add the following new claims:

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123. A method for identifying nucleic acid for target DNA sequences comprising:

a) co-encapsulating in a microenvironment DNA obtained from a mixture of organisms with a mixture of DNA probes comprising a detectable marker;

b) incubating the co-encapsulated mixture under such conditions and for such time as to allow hybridization of complementary sequences, thereby identifying target DNA sequences.

124. The method of claim 123, further comprising transforming host cells with target DNA to produce an expression library of a plurality of clones.

125. The method of claim 123, wherein the organisms are microorganisms.

126. The method of claim 125, wherein the microorganisms are uncultured microorganisms.

127. The method of claim 125, further comprising screeing the expression library for a specified enzyme activity.

128. The method of claim 123, wherein the target DNA obtained from the mixture of organisms is selected by:

a) converting double stranded DNA into single stranded DNA;

b) recovering from the converted single stranded DNA, single stranded target DNA which hybridizes to probe DNA;

c) converting recovered single stranded target DNA to double stranded DNA; and

d) transforming a host cell with the double stranded DNA of c).

129. The method of claim 123, wherein screening is by FACS analysis, a magnetic field sensing device, flow cytometry or a mass spectroscopic screening apparatus.

130. The method of claim 123, wherein said target DNA is gene cluster DNA.

131. The method of claim 125, wherein the microorganisms are derived from an environmental sample.

132. The method of claim 126, wherein the uncultured microorganisms comprise a mixture of terrestrial microorganisms or marine microorganisms or airborne microorganisms, or a

mixture of terrestrial microorganisms, marine microorganisms and airborne microorganisms.

133. The method of claim 124, wherein the clones comprise a construct selected from phage, plasmids, phagemids, cosmids, fosmids, viral vectors, P1 vectors or artificial chromosomes.

134. The method of claim 123, wherein the target DNA comprises one or more operons, or portions thereof, of the DNA population.

135. The method of claim 134 wherein the operon or portions thereof encodes a complete or partial metabolic pathway.

136. The method of claim 136, wherein the uncultured microorganisms comprise extremophiles.

137. The method of claim 135, wherein the extremophiles are selected from the group consisting of thermophiles, hyperthermophiles, psychrophiles, barophiles, and psychrotrophs.

138. The method of claim 128, wherein the host cell is selected from a bacterium, fungus, plant cell, insect cell or animal cell.

139. The method of claim 123, wherein the target DNA encodes a protein.

140. The method of claim 139, wherein the protein is an enzyme.

141. The method of claim 140, wherein the enzyme is selected from the group consisting of oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases.

142. The method of claim 123, wherein the microenvironment is a liposome, gel microdrop, bead, agarose, cell, ghost red blood cell or macrophage.

143. The method of claim 142, wherein the liposomes are prepared from one or more phospholipids, glycolipids, steroids, alkyl phosphates or fatty acid esters.

144. The method of claim 143, wherein the phospholipids are selected from lecithin, sphingomyelin or dipalmitoyl.

145. The method of claim 143, wherein the steroids are selected from cholesterol, cholestanol or lanosterol.

146. The method of claim 123, wherein the detectable marker is a fluorescent dye, a visible dye, a bioluminescent material, a chemiluminescent material, a radioactive material, or an enzymatic substrate.

147. The method of claim 146, wherein the bioluminescent material is green fluorescent protein (GFP) or red fluorescent protein (RFP).

148. The method of claim 147, wherein detection of the fluorescent dye or a visible dye is carried out by fluorometric or spectrophotometric measurement.

149. A method of identifying a molecule that affects cell growth comprising co-encapsulating the molecule and a target cell in a microenvironment under conditions that allow the

molecule and the target cell to interact, and detecting an effect on the growth of the target cell, thereby identifying a molecule that affects cell growth.

150. The method of claim 149, wherein the molecule is expressed in a cell and the cell is co-encapsulated in the microenvironment with the target cell.

151. The method of claim 149, wherein the effect is inhibition of cell growth.

152. The method of claim 149, wherein the effect is stimulation of cell growth.

153. The method of claim 149, wherein the effect is termination of all cell growth.

154. The method as in any of claims 55 or 149, wherein the molecule is produced by a *Streptomyces* or *Actinomyces* species.

155. The method of claim 154, wherein the *Streptomyces* or *Actinomyces* species are single cells or fragmented mycelia. --